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THE CYTOTAXONOMY OF THE *ASPLENIUM SPLENDENS* COMPLEX IN SOUTH AFRICA

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ABSTRACT

Morphological, ecological and cytological studies of *A. splendens* Kze. and related material in South Africa have shown it to be a polyploid species complex. *A. splendens* subsp. *splendens* is a tetraploid (n=72) while some closely related octoploid (n=144) material is referred to *A. multiforme* Kr., a species which has not been recognised in recent works on the fern flora of South Africa. A second tetraploid from the Drakensberg Mountains, which has previously been confused with *A. aethiopicum* (Burm) Bech. s.l., is also placed in the complex as a result of hybridisation experiments involving *A. splendens* subsp. *splendens* and *A. aethiopicum*. It may be regarded as a member of the same ecospecies as *A. splendens* and has been described for the first time as *A. splendens* subsp. *drakensbergense*. Analysis of the meiotic pairing in hybrids synthesised between the three members of the complex suggest that *A. multiforme* is an intervarietal autopolyploid which has arisen by hybridisation between the two subspecies of *A. splendens* followed by doubling of the chromosome number.

UITTREKSEL

DIE SITOTAKSONOMIE VAN DIE *ASPLENIUM SPLENDENS* KOMPLEKS IN SUID-AFRIKA.

Morfologiese, ekologiese en sitologiese studies van *Asplenium splendens* Kze. en verwante materiaal in Suid-Afrika het getoon dat dit 'n poliploïede spesies kompleks is. *A. splendens* subsp. *splendens* is 'n tetraploïed (n=72) terwyl sommige naby verwante oktoploïde (n=144) materiaal as *A. multiforme* Kr. bekend staan ('n spesies wat nie in die nuutste werke oor die varing flora van Suid-Afrika erken word nie). 'n Tweede tetraploïede vanaf die Drakensberge wat voorheen met die *A. aethiopicum* (Burm) Bech. s.l., verwar was, is ook as 'n resultaat van kruisings eksperimente met *A. splendens* subsp. *splendens* en *A. aethiopicum* in die kompleks geplaas. Dit mag beskou word as 'n lid van dieselfde ektospesies as *A. splendens* en was al vir die eerste keer beskryf as *A. splendens* subsp. *drakensbergense*. 'n Oorsig van die meiotiese paring in basters saamgestel uit die drie lede van die kompleks stel voor dat *A. multiforme* 'n tussen variëteits outopoliploïed is wat uit verbastering tussen die twee subspesies van *A. splendens* ontstaan het gevolg deur verdubbeling van die kromosoomgetalle.

INTRODUCTION

Asplenium splendens Kze. was originally described in 1836 from some South African specimens collected by Ecklon from the Kat River and by Drège from the lower reaches of the Umzimvubu River (Port St. Johns). A second closely related species, *A. multiforme* Kr., was subsequently described in 1900 from a Krook collection from Newmarket. Sim (1915) in the "Ferns of South Africa" did not recognise either of these taxa and erroneously placed them both under *A. cuneatum* Lam. Later Alston and Schelpe (1952) and Schelpe (1969) segregated the South African material under *A. splendens* but they did not distinguish *A. multiforme*.

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Recent cytological studies have revealed two different chromosome numbers within *A. splendens* and have confirmed the presence of a species complex. Typical *A. splendens* is a tetraploid species with 72 chromosomes at meiosis whilst a plant from the eastern Cape has shown twice this number and is therefore octoploid. The octoploid material agrees in all respects with the specimen described as *A. multiforme* and, since it can be distinguished from the tetraploid material by careful morphological examination, its specific rank has been restored. Some further tetraploid material from the Drakensberg Mountains has also been included in the investigation because of its morphological similarity to *A. multiforme*. This material has in the past been loosely referred to *A. aethiopicum* (Burm.) Bech. s.l. and not recognised as part of the present complex, but during the course of the experimental studies it became evident that it is closely related to *A. splendens* and *A. multiforme*. It is, however, sufficiently different from typical *A. splendens* to merit taxonomic recognition and on the basis of the present experimental evidence it is recognised as a subspecies of *A. splendens* for which the name *drakensbergense* is proposed.

Experimental hybrids have been synthesised between the three representatives of the complex and meiosis studied in the hybrids in an attempt to analyse their inter-relationships. The hybridisation programme has also been extended to incorporate a representative of *A. aethiopicum* but this species is only included in the present communication to the extent required to interpret relationships within the *A. splendens* complex.

The observations recorded substantially clarify the taxonomic position of the material described as *A. splendens* subspecies *drakensbergense* and permit some conclusions to be reached on the relationships of the three members of the complex and, in particular, on the origin of the octoploid *A. multiforme*.

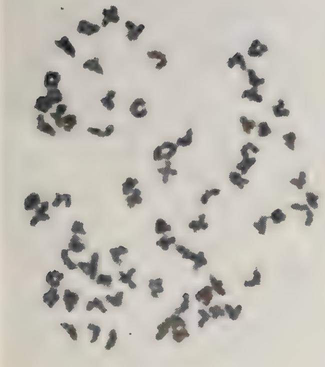
PLATE I.

1, 2 and 3 Permanent acetocarmine squash preparations of chromosome pairing at meiosis (X1000).

1. Tetraploid hybrid between *A. splendens* subsp. *splendens* B 71, Pirie Forest and *A. splendens* subsp. *drakensbergense* Edwards s.n., Impendhle, showing regular chromosome pairing with 72 bivalents.
2. Tetraploid hybrid between *A. splendens* subsp. *splendens* B 71, Pirie Forest and *A. aethiopicum* McNeil s.n. Transvaal showing complete failure of chromosome pairing with 144 univalents.
3. Hexaploid hybrid between *A. multiforme* B 108, Askeaton and *A. splendens* subsp. *splendens* B 71, Pirie Forest showing 72 paired groups (trivalent and bivalent) and 51 univalents. For an explanatory diagram see fig. 5.

4, 5 and 6. Spores photographed in gum chloral (X250).

4. *A. splendens* subsp. *splendens* B 259, Woodbush.
5. *A. splendens* subsp. *drakensbergense* Edwards s.n., Impendhle.
6. *A. multiforme* B 108, Askeaton.



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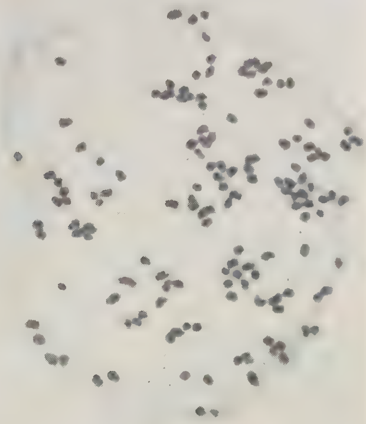

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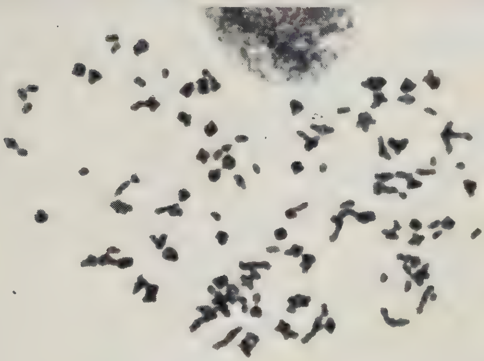

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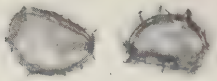

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THE MATERIAL

The living plants which have been available during the investigation are listed in Appendix I with details of their locality, collector, chromosome number and spore size. All the plants have been successfully established in cultivation at the Botany Departments, first of the University of Leeds, and later, of the University of Nottingham. The material has been derived as living sporophytes either donated by correspondents in South Africa or collected by the author during fieldwork in South Africa between July 1960 and June 1961.

The following systematic treatment of the material is based on all the morphological, ecological and cytological observations and on the experimental results which are discussed in the following sections. It is placed here to provide a nomenclatural basis for the experimental work and also to orientate the reader so that he will be better able to evaluate the experimental data.

1a *A. splendens* Kze subsp. *splendens*

A. splendens Kze Linn. 10, 516, 1836.

The material attributed to this subspecies possesses ovate-acuminate to sometimes nearly deltoid fronds up to 75 cm. long, which rise at intervals of $\frac{1}{2}$—1 cm. from a creeping rhizome covered with light brown clathrate scales. The fronds are bipinnate to tripinnate with 9—16 pairs of pinnae which are subdivided into as many as 14 cuneate rounded trapezioid pinnules. The larger pinnules are often divided again into cuneate rounded segments. The stipe is black or dark brown and the rachis is also dark on the undersurface for the greater part of its length.

All the living specimens which have been cytologically examined have proved to be tetraploid with 72 bivalents at meiosis (see Appendix I) and comparable pinnae from six of these plants are illustrated in fig. 1, a–f and rhizome scales from two representatives in fig. 2, a and b.

Although cytologically uniform, subspecies *splendens* shows considerable morphological variation particularly with regard to frond dissection and the shape of the pinnules. Fronds vary from a bipinnate condition with entire cuneate rounded pinnules to a fully tripinnate condition, where the lowermost pinnules are elongate and divided into several cuneate rounded segments. The latter form has been recognised as a variety by Kunze (1836) and Sim (1915). There is, however, a continuous gradation between the two extremes of frond dissection, sometimes on the same plant (see fig. 1, e and f), so that the separation of the more dissected usually larger form as a variety would be arbitrary and superfluous.

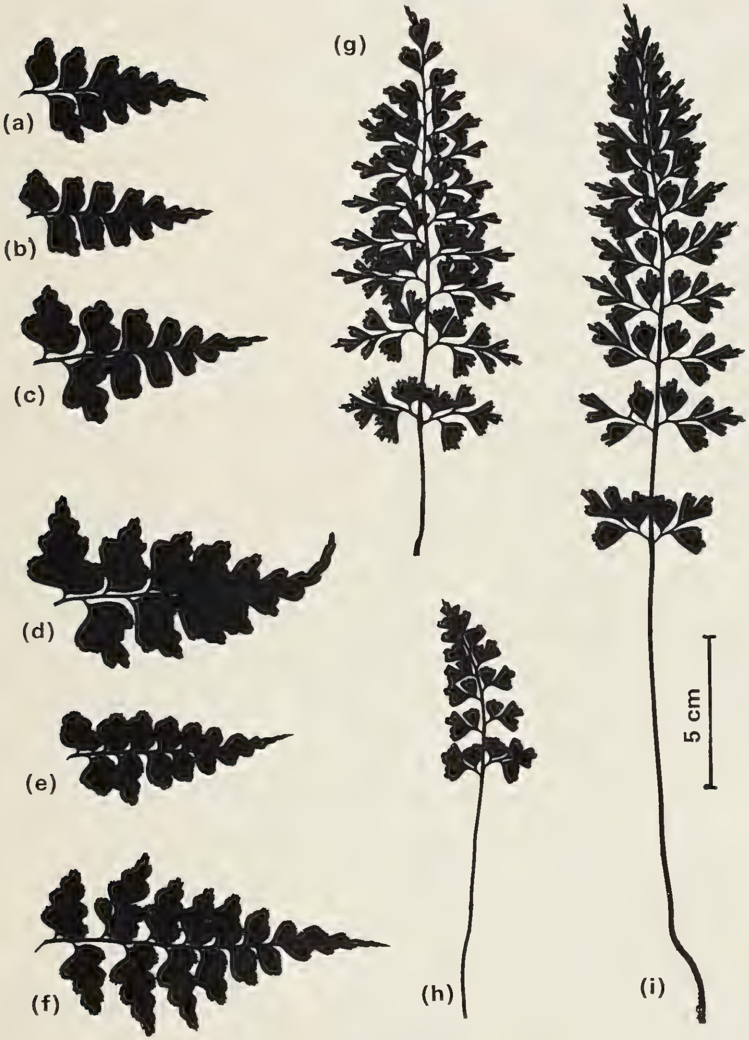

FIG. 1.

Silhouettes of pinnae and fronds of *A. splendens*. All half natural size. a), b), c), d), e), and f) subspecies *splendens*. Comparable pinnae from cultivated plants. a) B 41, Ngome Forest. b) B 59, Oribi Gorge. c) B 71, Pirie Forest. d) B 249 Magoebas Kloof. e) and f) B 259, Woodbush. g), h) and i) subspecies *drakensbergense* Edwards s.n. Impendhle. g) frond from cultivated plant h) and i) wild material. For full details of locality see Appendix I.

Much of the variation is a function of the age and condition of the plants as well as of ecological factors. Juvenile and small plants growing in open drier habitats generally possess entire cuneate pinnules with conspicuously rounded apices; while mature plants growing luxuriantly in moist shaded conditions are often tripinnate with markedly elongate and divided pinnules. Nevertheless even after several years in cultivation under similar conditions some variation persists in mature fronds as shown by the pinnae in fig. 1, a–f.

A. *splendens* subsp. *splendens* is primarily a forest taxon at altitudes ranging from 80—5 000 feet. It is usually seen in shade on the forest floor or on mossy boulders or more occasionally as a low level epiphyte, but it does extend beyond the present forest range particularly in the eastern Cape and Natal. In these areas it may be found in drier bush and wooded kloofs where it sometimes shows appreciable drought resistance. These 'outliers' away from the main forest areas may represent part of a relict flora from the time when the forested areas were more extensive than they are today. The distribution extends from the Albany Division in the Cape Province to the Zoutpansberg in the northern Transvaal (fig. 6).

1b. **A. *splendens* subspecies drakensbergense,** A. Braith. subsp. nov.

Rhizoma breve repens, paleis brunneis clathratis concoloribus lineari-subulatis, c. 5 mm longis, $\frac{1}{2}$ mm latis, margine projecturis interdum filiformibus munitis. *Stipites* 6—19 cm longi, laminis aliquando aequantes vel longiores, inferne atrobrunnei supra viridescentes, paleis eis rhizomatis similibus sed parvioribus sparse instructi. *Lamina* 5—16 cm longa, 2—5 cm lata, oblongo-lanceolata ad anguste ovata vel interdum anguste triangularis, bipinnata; rachide viride, paleis et fibrillis sparse instructa. *Pinnae* 6—13 -jugae subоppositae, usque ad 3 cm longae, 2 cm latae, trapeziformes, 1—4 pinnulis infra apicem lobatum. *Pinnulae* cuneatae apicibus truncatis irregulariter inciso-serratis, glabrae, imae saepe lobatae. Lobatae pinnarum et pinnularum oblongo-rectangulatae, apicibus truncatis. *Sori* usque ad 6 mm longi, versus basin pinnularum vel loborum pinnarum dispositi. *Sporae* brunneae, 33—48μ longae (ordo mediis 38—42μ); perispora spissate papillosa, cristis undulatis tenuibus 4—6μ latis anastomosantibus. Chromosomatum numerus n = 72; reproductio sexualis.

TYPE: Impendhle, Natal, South Africa. Nr. waterfall c. 5 000′. June 1961. *Edwards* s.n; living plant collected at the same time and cultivated at Nottingham. (BM, holotype; BOL, Herb. Braithwaite, isotypes).

Rhizome short creeping, covered with brown clathrate concolorous linear-subulate scales, c. 5 mm long, $\frac{1}{2}$ mm wide, bearing occasional filiform projections on the margin. *Stipes* 6—19 cm long, sometimes equalling or longer than the lamina, dark below becoming green above and bearing a few scales similar to,

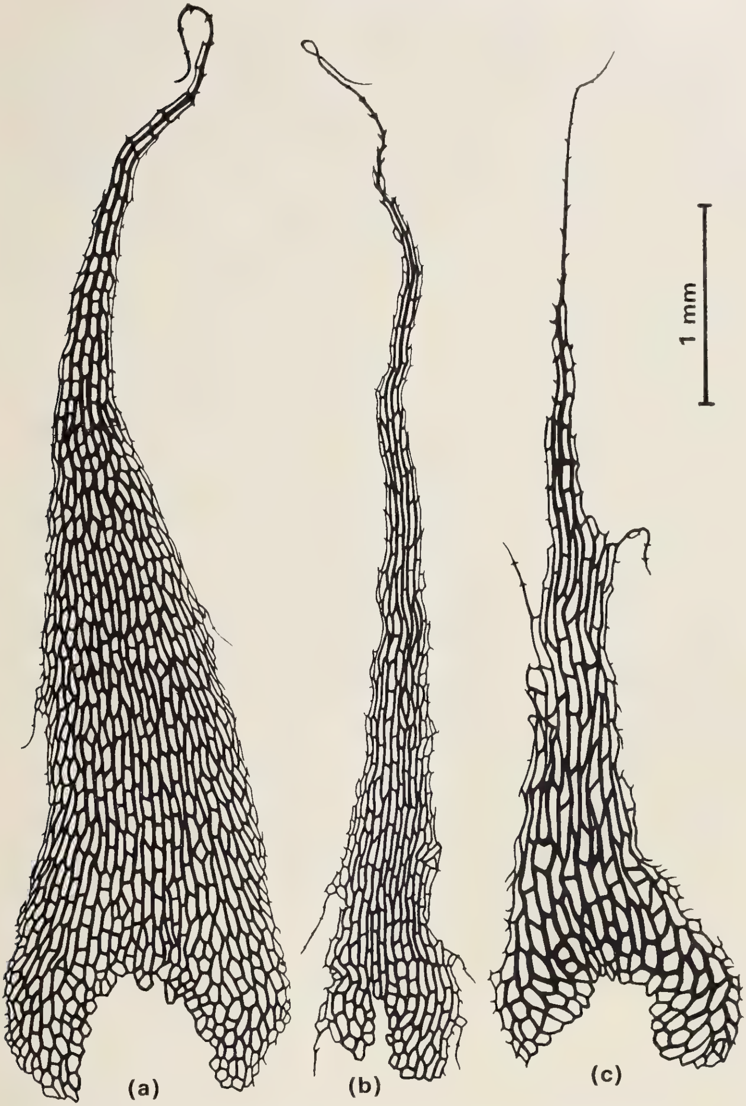

FIG. 2.
Rhizome scales of *A. splendens*.
a) subspecies *splendens* B 259, Woodbush. b) subspecies *splendens* B 71, Pirie Forest. c) subspecies *drakensbergense* Edwards s.n., Impendhle.

but smaller than, those of the rhizome. *Lamina* 5—16 cm long, 2—5 cm wide, oblong-lanceolate to narrowly ovate or occasionally narrowly triangular, bipinnate; rachis green, sparsely bearing a few scales and fibrils. *Pinnae* 6—13 subopposite pairs, up to 3 cm long, 2 cm wide, trapeziforme, with 1—4 pinnules below a lobed apex. *Pinnules* cuneate with truncate irregularly inciso-serrate apices, glabrous, the lowermost often lobed. Lobes of pinnae and pinnules oblong-rectangular with truncate apices. *Sori* up to 6 mm long and situated towards the base of pinnules and pinnae lobes. *Spores* brown, 33—48μ long (range of means 38—42μ); perispore densely papillose with a thin anastomising undulating wing 4—6μ wide. Chromosome number $n = 72$ (see Appendix I); reproduction sexual.

Silhouettes of three fronds from the type material are shown in fig. 1, g–i and a rhizome scale and the spores in fig. 2, c and Plate I, 5 respectively. A list of herbarium specimens attributed to subspecies *drakensbergense* may be found in Appendix II. It is characteristically a fern of rock crevices, rocky banks or screes at altitudes of 5 000 to 9 000 feet and at present is known only from Lesotho and the Natal Drakensberg (fig. 6).

The material described here as subspecies *drakensbergense* has previously been confused with *A. aethiopicum* s.l. but it can be readily distinguished from the latter species by its markedley cuneate truncate pinnules and by the absence of small scales and fibrils from the undersurfaces of the pinnae except for a few on the costa. It differs from subspecies *splendens* by its darker brown rhizome scales which are made up of larger cells (fig. 2), by its smaller narrower fronds with a green rachis and with less dissected pinnae bearing cuneate straight-sided truncate pinnules and by its larger and darker brown spores (Appendix I, Plate I, 4 and 5). In addition subspecies *drakensbergense* is generally found at higher altitudes than subspecies *splendens*.

2. *A. multiforme* Kr. Ann. des K. and K. Nat. Hof., **15:** 1 (1900).

This species was first described from a specimen collected by Krook from Newmarket (N. of Kokstad, Mt. Currie Div., Cape Prov.) and the octoploid material from Askeaton (Appendix I, fig. 3) agrees well with the type specimen in all respects including spore size.

Sim (1915) reduced *A. multiforme* to synonymy under his *A. cuneatum* Lam. and subsequent workers have included the material under *A. splendens* (subspecies *splendens*). It is altogether a smaller plant than *A. splendens* subspecies *splendens* with darker brown rhizome scales which are made up of much larger cells (fig. 2). The fronds are thicker in texture and the smaller pinnae are much less dissected and bear more compact rounded pinnules which are usually entire with more or less truncate apices. The spores are much larger and slightly darker (Appendix I, Plate I, 6).

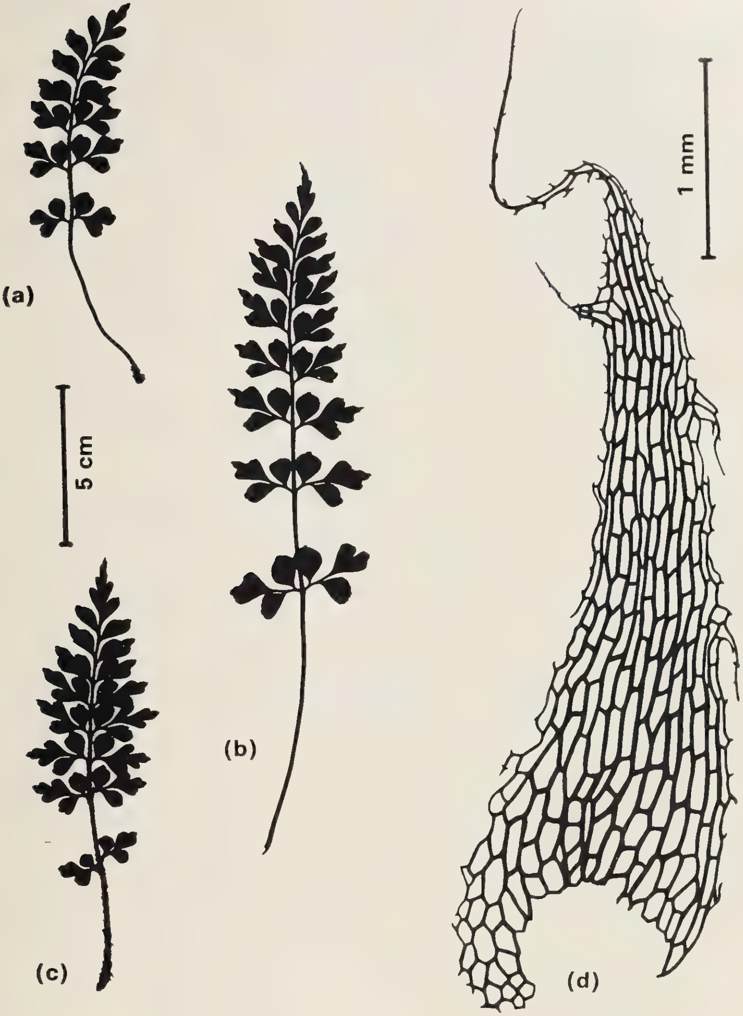

Fig. 3.

Silhouettes of fronds and a rhizome scale of *A. multiforme*. Fronds half natural size.
a) and b) wild material, B 108, Askeaton. c) Frond from cultivated plant B 108 Askeaton.
d) Rhizome scale B 108 Askeaton.

A. multiforme also differs ecologically from *A. splendens* subspecies *splendens.* While the latter is basically a forest taxon, *A. multiforme* is a rock crevice fern of drier areas. It is usually associated with rocky outcrops, often doleritic, in open grassland and bush areas sometimes growing with *Aloe, Euphorbia* and *Crassula* species. In the locality near Askeaton the plants were almost completely dried up in November and could easily have been overlooked. Other ferns in the same locality included representatives of some of the 'drier' country genera such as *Pellaea, Notholaena* and *Cheilanthes.*

The only other taxa likely to be confused with *A. multiforme* is *A. splendens* subspecies *drakensbergense* but the latter can be distinguished by its longer narrower fronds with a completely green rachis and by its more dissected pinnae bearing cuneate straight-sided pinnules. The spores are also smaller than those of *A. multiforme* (Appendix I, Plate I, 5 and 6).

The morphological, ecological and cytological differences between this material and *A. splendens* it is felt are sufficient to justify restoring its specific status.

A list of herbarium material which is attributed to *A. multiforme* may be found in Appendix II and the distribution is shown in fig. 6.

THE HYBRIDISATION PROGRAMME

The following wild gatherings have been used in the hybridisation experiments:

1) *A. splendens* subspecies *splendens.* 4x, Braithwaite 71, Pirie Forest, King Williams Town, Cape Province.
2) *A. splendens* subspecies *drakensbergense* 4x, Edwards s.n., Impendhle, Natal.
3) *A. multiforme* 8x Braithwaite 108, Nr. Askeaton, Xalanga Div., Cape Province.
4) *A. aethiopicum* s.l. 4x McNeil s.n., Transvaal.

The procedures used in the hybridisation programme have been fully described by Lovis (1969) and need not be repeated here. Chromosome preparations have been made using the acetocarmine squash method for fern spore mother cells described by Manton (1950).

The results of the hybridisation programme, giving details of the hybridisations attempted and the cytological information from the successful combinations, are assembled in Table I.

At the tetraploid level it is immediately evident from the percentage success data in Table I (items 1–3) that, while it is relatively easy to synthesise hybrids

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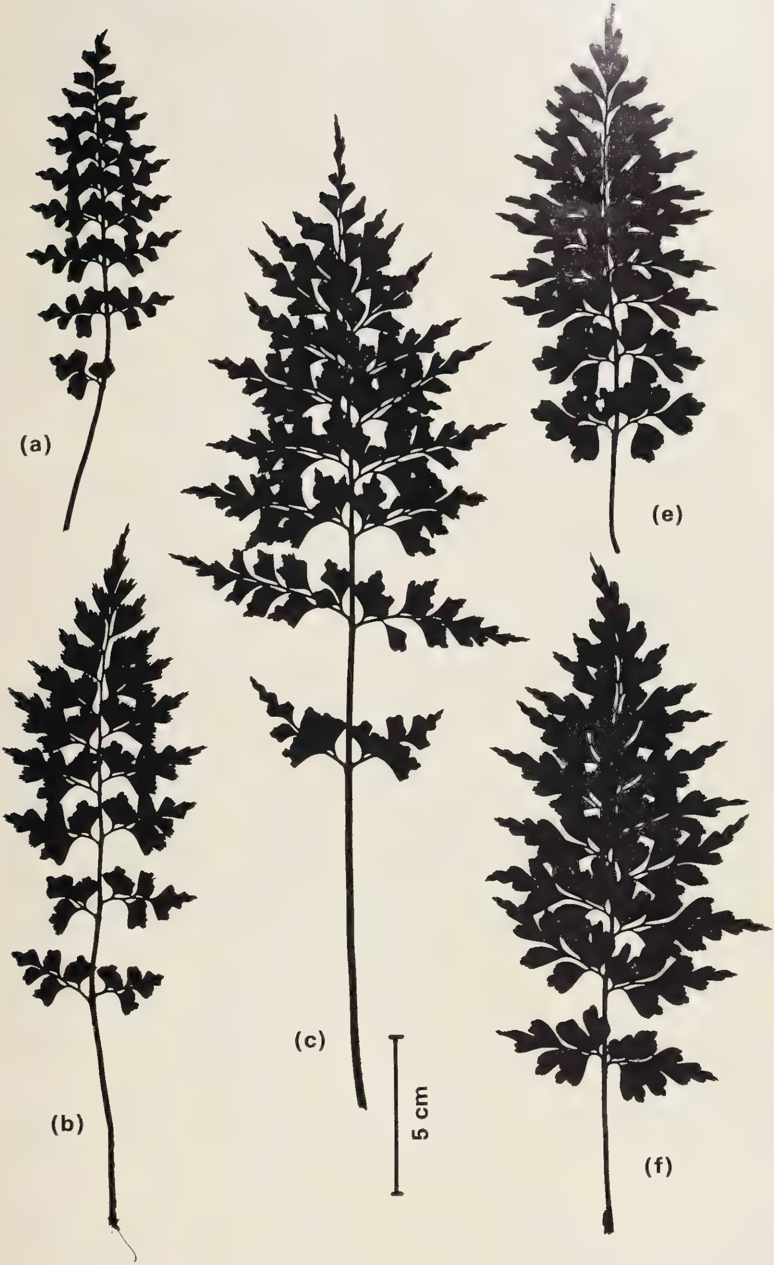
FIG. 4.

Silhouettes of fronds from tetraploid hybrids between the subspecies of *A. splendens*. All half natural size. a), b) and c) ♀ subspecies *drakensbergense* Edwards s.n., Impendhle X ♂ subspecies *splendens* B 71 Pirie Forest. Three fronds from hybrid AB 648A.

d) and e) Reciprocal cross. Fronds from young plants. d) AB 571A. e) AB 571B.


(a)
(b)
(c)
(e)
(f)
5 cm

between the two subspecies of *A. splendens*, it is much more difficult to combine either of these subspecies with tetraploid *A. aethiopicum* from South Africa. In fact it has so far not been possible to synthesise a hybrid between *A. splendens* subsp. *drakensbergense* and *A. aethiopicum*.

TABLE 1

Details of hybridisation experiments involving *A. splendens*, *A. multiforme* and tetraploid *A. aethiopicum*.

	No. of prothalli	*No. of hybrids*	*%age success*	*Chrom. pairing at meiosis*
Tetraploid hybrids 1) ♀ A. splendens subsp. splendens X ♂ A. aethiopicum 4x. Reciprocal hybrid	50 20 } 70	1 7 } 8	11%	Virtually no pairing. 144 univalents
2) ♀ A. aethiopicum 4x X ♂ A. splendens subsp. drakensbergense.	48	0	0%	– – – –
3) ♀ A. splendens subsp. drakensbergense. X ♂ A. splendens subsp. splendens. Reciprocal hybrid	16 32 } 40	13 17 } 30	75%	Complete pairing. 72 bivalents.
Hexaploid hybrids 4) ♀ A. multiforme X ♂ A. splendens subsp. splendens.	8	5	63%	71–72 paired groups (trivalent & bivalent) + 48–54 univalents.
5) ♀ A. multiforme X ♂ A. splendens subsp. drakensbergense.	22	12	55%	70–72 paired groups (trivalent & bivalent) + 45–51 univalents.

The tetraploid hybrids between the two subspecies of *A. splendens* were vigorous and morphologically intermediate between the two parents although the characters of the female parent tended to predominate so that the reciprocal hybrids differed slightly (fig. 4). Two hybrids synthesised using subspecies *drakensbergense* as the female parent and one sythesised in the other direction have been cytologically examined. In each case meiosis was regular with 72 bivalents. The hybrids produced well filled spores and those from one plant which has been tested have shown good germination, but an F2 generation has not yet been raised.

In contrast the tetraploid hybrids between *A. splendens* subspecies *splendens* and *A. aethiopicum* show virtually no chromosome pairing at meiosis. In two hybrids examined in detail the chromosomes appeared as 144 univalents and the plants were almost completely sterile except for a few apparently well filled spores.

The two different types of meiotic behaviour found in the tetraploid hybrids are illustrated in Plate I, 1 and 2.

The hexaploid hybrids (items 4 and 5 in Table I) involving *A. multiforme* and the two subspecies of *A. splendens* are relatively easily synthesised; the hybridisation attempts for both crosses being more than 50% successful. The hybrids were vigorous but sterile and two representatives of each cross have been investigated cytologically.

The chromosome pairing in each of the hexaploid combinations is essentially similar so that their cytology may be conveniently considered together. A representative cell from the hybrid between *A. multiforme* and *A. splendens* subspecies *splendens* is shown in Plate I, 3 and an analysis of the same cell is shown in fig. 5. This cell shows 72 paired groups (21 trivalents + 51 bivalents) and 51

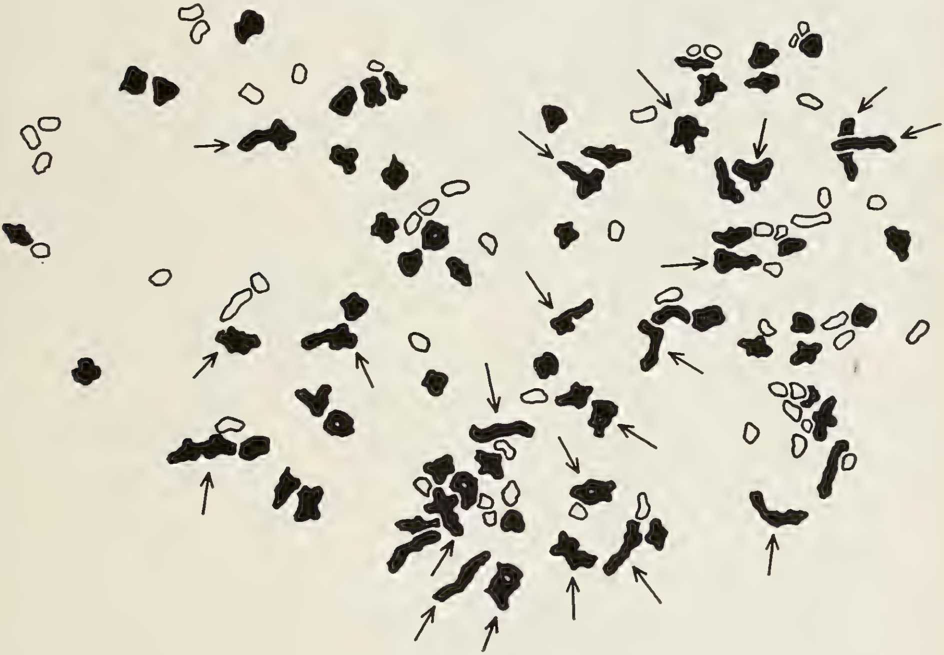


FIG. 5.

Chromosome pairing in a hexaploid hybrid between *A. multiforme* B 108, Askeaton and *A. splendens* subsp. *splendens* B 71, Pirie Forest. Explanatory diagram for Plate I, 3 showing 21 trivalents in black and arrowed, 51 bivalents in black and 51 univalents in outline. (X 1500).

univalents. The analyses obtained for both hybrid combinations fall into the range of 70—72 paired groups and 45—54 univalents. In all the cells analysed there are a significant number of trivalents, although it is not always easy to identify them with complete accuracy when dealing with chromosome numbers as high as in these hybrids. However, the number of univalents in all the cells analysed is significantly much lower than 72 (the number which would be expected if all the paired groups were bivalent) which indicates that between 18 and 27 of the paired groups are tricelent.

INTERPRETATION AND DISCUSSION OF THE OBSERVATIONS

It is evident from the percentage success data of the hybridisation attempts at the tetraploid level in Table I that there are considerable differences in the compatibility on the one hand, between the two subspecies of *A. splendens* and on the other, between these two subspecies and tetraploid *A. aethiopicum*. These differences suggest that the affinities of the material described as *A. splendens* subspecies *drakensbergense* do not lie with *A. aethiopicum* with which it has been confused, but rather with *A. splendens*.

The regular chromosome pairing observed in the hybrids between the two subspecies of *A. splendens* provides further evidence of their close relationship, although the 72 bivalents appearing in hybrids can be interpreted in two ways. The 72 chromosomes contributed by the gametes of each parent may be sufficiently homologous to allow them to pair completely and form 72 bivalents in the hybrid. Alternatively, the 72 chromosomes contributed by each parent may be completely non-homologous but are capable of pairing among themselves (i.e. autosyndetic pairing). In this case the chromosomes of each parental gamete contribute 36 bivalents to the total of 72 bivalents found in the hybrid; thus resembling the cytological behaviour of certain European tetraploid hybrids in *Asplenium* (Lovis, 1964). However, the virtual lack of any chromosome pairing in the hybrids between *A. splendens* and *A. aethiopicum* indicates that the chromosomes of subspecies *splendens* are not capable of any appreciable autosyndesis. The only interpretation, therefore, which is consistent with the facts as they are at present known is that the chromosomes of the two subspecies are essentially homologous and are capable of pairing completely in the hybrids to form 72 bivalents.

On the other hand the virtual absence of any chromosome pairing in the tetraploid hybrids involving tetraploid *A. aethiopicum* strongly suggests that there is little or no homology between the chromosomes of *A. splendens* and *A. aethiopicum*.

The combined evidence from the tetraploid hybrids thus emphasises the distinctness of *A. aethiopicum* from *A. splendens* and confirms a close relation-

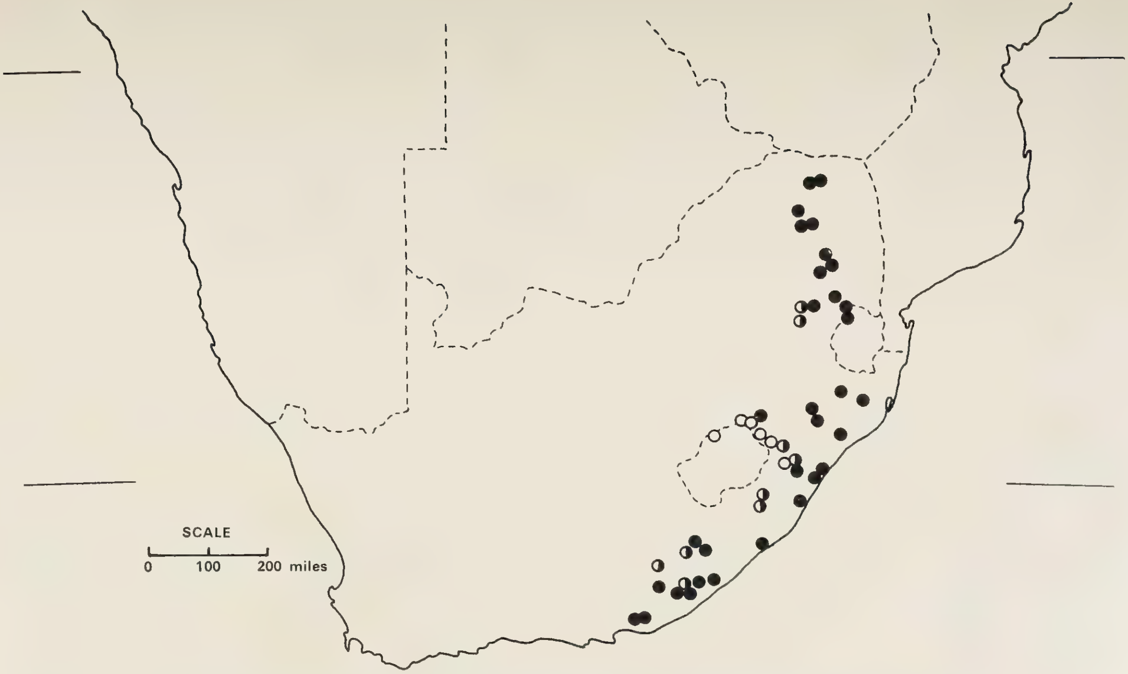


FIG. 6.

Map to show the distribution of *A. splendens* subsp. *splendens* (black circles), *A. splendens* subsp. *drakensbergense* (open circles) and *A. multiforme* (half black circles).

ship of the material treated here as *A. splendens* subspecies *drakensbergense* and *A. splendens* subspecies *splendens.*

There is at present little or no evidence for any cytogenetic differentiation which is sufficient to produce a sterility barrier within *A. splendens* at the tetraploid level. The F1 hybrid between the two tetraploid forms is fully fertile as far as can be judged from spore output and spore germination. The two tetraploids, therefore, may be regarded as members of the same ecospecies (Turesson, 1922, 1929) and, in consequence, have been treated as members of the same taxonomic species. Nevertheless there has been considerable morphological and ecological differentiation between the two tetraploids which merits taxonomic recognition at the infraspecific level. The two taxonomic subspecies which have been recognised may well represent morphologically distinct ecotypes, but before their precise relationship can be determined it is clearly desirable to raise an F2 generation.

Turning next to the cytology of the hexaploid hybrids (items 4 and 5, Table I) involving *A. multiforme*, the meiotic pairing for both crosses falls within the range of 70—72 paired groups (including 18—27 trivalents) and 45—54 univalents. The pairing in these hybrids is open to alternative interpretation, but in view of the close morphological relationship of the parental taxa it seems reasonable to suppose in both cases that maximum pairing is occurring between the 72 chromosomes contributed by the tetraploids and 72 of the 144 chromosomes of *A. multiforme.* If this is the case then any pairing in excess of 72 bivalents can only be accounted for by autosyndetic pairing among the chromosomes contributed by *A. multiforme.* Thus the presence of 18–27 trivalents reflects the minimum number of pairing homologies which exist in the chromosome complement of the octoploid. The full extent of the autosyndetic capacity of the chromosomes of *A. multiforme* cannot be ascertained from the present hybrids, but it is probably much greater than that indicated in the interpretation above since it is unlikely to be fully expressed as trivalents in these hexaploid hybrids. It can only be determined by either crossing *A. multiforme* with a totally unrelated species or by producing a tetraploid plant by inducing apogamy in the gametophyte of *A. multiforme.*

It is clear from the meiotic behaviour of the hexaploid hybrids that there is an equally close relationship between the two tetraploids and the octoploid and that there are considerable homologies among the gametic set of chromosomes of the octoploid. These homologies are sufficient to rule out the possibility of a true allopolyploid* origin for *A. multiforme* and suggest it is either an autopolyploid* (including intervarietal autopolyploid*) or a segmental allopolyploid* relative to the tetraploid genome.† On this interpretation, designating the haploid set of chromosomes of the tetraploid as A, the genomic constitution of *A. multiforme* can be expressed as AAAA where the genomes are completely homologous and capable of pairing completely under suitable conditions or AAA1A1 where the genomes A and A1 are differentiated to the extent where they are only partially homologous and not capable of pairing completely. It should however be emphasised that the precise autosyndetic capacity of *A. multiforme* remains to be demonstrated and until this is done it is not possible on the direct cytological evidence available at present to exclude either of these possibilities. It will be necessary to suspend judgement until other evidence on the origin of *A. multiforme* has been considered.

A. multiforme is morphologically somewhat intermediate between *A. splendens* subsp. *splendens* and subsp. *drakensbergense*. Its rhizome scales, frond size and texture, degree of dissection of the pinnae and its truncated pinnules all resemble subspecies *drakensbergense* more closely but it possesses in common with subspecies *splendens* rounded pinnules and a rachis which is dark on the undersurface for at least half its length. It also occupies an intermediate range of altitude from 2 600—5 800 feet, while subspecies *splendens* is found from 80—5 000 feet and subspecies *drakensbergense* from 5 000—9 000 feet, so that its distribution tends to lie between those of the two tetraploids (fig. 6). These facts would seem to preclude a strict autopolyploid origin of *A. multiforme* from a single tetraploid type and indicates that both tetraploids have been involved in the origin of the octoploid. Since the two tetraploids contain essentially homologous chromosomes and apparently belong to the same ecospecies, such an origin would suggest that *A. multiforme* is an intervarietal autopolyploid rather than a segmental allopolyploid. There is, therefore only one conclusion which is consistent with morphological and ecological evidence and with the cytological data from the hybrids, namely, that *A. multiforme* is an intervarietal autopolyploid which has arisen by the doubling of the chromosome complement of a hybrid or hybrid segregate between the two morphologically and ecologically differentiated subspecies of *A. splendens*.

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* The classification of polyploids into four main types—*autopolyploids*, *segmental allopolyploids*, *true* or *genomic allopolyploids* and *autoallopolyploids*—by Stebbins (1947, 1950) is being adopted here. By *autopolyploid* is meant a polyploid containing two or more pairs of genomes made up of completely homologous chromosomes which are capable of pairing completely under suitable conditions and produce fertility when present together in the diploid condition. This is further subdivided into *strict autopolyploids* which have arisen from a single diploid type and *intervarietal autopolyploids* which have arisen from fertile diploid hybrids between morphologically and ecologically differentiated diploid types. A *segmental allopolyploid* is a polyploid containing two or more pairs of genomes made up of completely or partially homologous chromosomes which are able to pair all or a substantial number of their chromosomes under suitable conditions, but the genomes are differentiated in respect of a sufficiently large number of genes, chromosome segments or whole chromosomes to produce sterility when in the diploid condition. *True* or *genomic allopolyploidy* denotes the presence of two or more pairs of genomes made up of non-homologous chromosomes which are so different that they are unable to pair in the diploid condition. An *autoallopolyploid* contains more than two pairs of genomes and combines the characteristics of autopolyploids and allopolyploids.

† The evolution of a complex would normally be considered in terms of the basic or monoploid chromosome number of the genus, which in Asplenium is 36 (Manton, 1950). However, no diploids with 36 chromosomes at meiosis are known in either the group under investigation or any of its relatives, so that its evolution can only be usefully discussed at present in terms of the lowest level of polyploidy—the tetraploid.

A. multiforme normally shows 144 bivalents at meiosis although one spore mother cell has been observed with a single trivalent and an unpaired chromosome. The almost complete absence of multivalents from the wild material would suggest that its autosyndetic capacity is normally being kept in check by a genetical mechanism suppressing the formation of multivalents. It is now well established that meiosis is subject to genic control and it is not difficult to visualise such a mechanism appearing in natural populations where selection pressure is favouring increased fertility. The diploidised condition of *A. multiforme* would be in agreement with that found in a number of polyploids in the flowering plants and ferns, including several tetraploid *Aspleniums* (Lovis, 1964; Lovis et al, 1969; Lovis and Reichstein, 1969), which are known to be autopolyploids but form only bivalents at meiosis.

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APPENDIX I

Details of the material which has been cytologically examined.

Locality	*Collector and Number*	*Cytology*	*Spore size*
A. splendens Kze subsp. splendens			
Transvaal, S. Africa	McNeil s.n.	$n = 72$	
Ngome Ft., Ngotshe Div., Natal, S. Africa	Braithwaite 41	$n = 72$	33,2 ± 2,54
Oribi Gorge, Port Shepstone Div., Natal, S. Africa	Braithwaite 59	$n = 72$	34,0 ± 2,27
Pirie Ft., King Williams Town, Cape, S. Africa	Braithwaite 71	$n = 72$	33,7 ± 2,32
Magoebas Kloof, Tzaneen, Pietersberg Div., Transvaal, S. Africa	Braithwaite 249	$n = 72$	33,4 ± 2,28
Woodbush Ft. Reserve, Pietersberg Div., Transvaal, S. Africa	Braithwaite 259	$n = 72$	34,9 ± 2,50
A. splendens Kze subspecies drakensbergense A. Braith. subsp. nov.			
Impendhle, Natal, S. Africa	Edwards s.n.	$n = 72$	40,3 ± 3,92
Ndedema area, Drakensberg, Bergville Div., Natal, S. Africa	Esterhuysen	$n = 72$	40,2 ± 3,95
(live plant collected at same time as Esterhuysen 28501, BOL)			
A. multiforme Kr.			
Nr. Askeaton, Xalanga Div., Cape, S. Africa	Braithwaite 108	$n = 72$	47,5 ± 3,90

Herbarium specimens of the material listed will be deposited at the British Museum (Natural History), London, and the Bolus Herbarium, University of Cape Town.

APPENDIX II

Herbarium specimens attributed to *A. splendens* Kze subspecies *drakensbergense* A. Braith. subsp. nov.

Natal: Impendhle, 5 200′, 2-vi-45. *Clarkson* 165. Mixed collection with *A. aethiopicum* s.l. (NH); Cathedral Peak area, Drakensberg, Weenen Div., shallow soil on rock in scree in kloofs, 7–8 000′, July 1949. *Esterhuysen* 15519 (BOL); Injasuti area, Drakensberg, Estcourt Div., rocky places, 5–6 000′, July 1956. *Esterhuysen* 26075 (BOL); Ndedema area, Drakensberg, Bergville Div., at base of rock, on slopes, on rocky banks and in recesses and screes, 7–9 000′, July 1960, *Esterhuysen* 28501 (BOL).

Lesotho: Banks of Caledon River, Leribe. *Dieterlen* 914 (PRE); without precise locality, 1861. *Cooper* 761 (PRE).

Herbarium specimens attributed to *A. multiforme* Kr.

Transvaal: Belfast. Under rocks along river. *Doidge* 29 (PRE); Carolina, c. 5 800′ *Rogers* 19730 (S–PB).

Natal: Vechtlager, Estcourt Div., Crest of rocky dolerite ridge. Crevice in boulders, c. 4 500′. 20-vii-44 *Acocks* 10502 (PRE, NH); Winters Kloof. In bush. *Doidge* s.n. (mixed collection) (PRE).

Cape: Stutterheim Commonage. Crevice in boulder. Rare. c. 2 600′, 19-iv-43 *Acocks* 9770 (PRE); Nr. Askeaton, Xalanga Div., Among rocks by roadside, c. 4 000′ 1-xi-60. *Braithwaite* 108 (BM, BOL); Nr. Askeaton, Xalanga Div., locally common in moist rock crevices on rock flush, c. 4 000′. 17-xii-55 *Schelpe* 5382 (BOL); Winston, Cathcart Div. Crevices in boulders on rocky dolerite ridge with Crassula. Locally common, c. 5 000′. *Acocks* 11396 (PRE); Kokstad, Mt. Currie Div. *Mogg* s.n. (PRE); Kamacho (locality unplaced) *Paterson* 646 (PRE).